

# **The forma *canteneri* STAUDINGER, 1861 of *Zerynthia rumina* (LINNAEUS, 1758), an interesting case of sex-linked inheritance: a study of its occurrence in captive-bred specimens and notes about other forms and aberrations**

(Lepidoptera, Papilionidae)

by

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**Abstract:** This paper reports on an investigation into the mode of inheritance of the rare f. *canteneri* STGR. of *Zerynthia rumina* (L.) and examines the theoretical predictions of its sex-linked inheritance's model. In the Lepidoptera, ♀ chromosomes are heterogametic XY (also referred to as WZ) and ♂ homogametic XX (or WW). A study of the inter-year comparisons of the offspring of this butterfly obtained in captivity indicates that the occurrence of f. *canteneri* STGR appears to follow a model of inheritance for a recessive allele (a mutation in a single gene, sex-linked trait), a single locus on the Y chromosome (Y-linked gene) that is probably missing from the X chromosome or is present but suppressed. In contradiction to the opinion of other authors, this would explain the absence of f. *canteneri* STGR in the ♂♂ of this species, a similar occurrence referring to ♀♀ of *Papilio glaucus* (LINNAEUS, 1758) in North America. Finally, we show further interesting forms and aberrations of *Z. rumina* (L.) obtained from our experiences of rearing in captivity.

**Resumen:** Este artículo informa sobre una investigación acerca del modo de herencia de la rara f. *canteneri* STGR. de *Zerynthia rumina* (L.) y examina las predicciones teóricas de su modelo de herencia ligado al sexo. En Lepidoptera, las ♀♀ son heterogaméticas XY (también nombrado WZ) y los ♂♂ homogaméticos XX (o WW). El estudio y las comparaciones interanuales de la descendencia obtenida de esta mariposa en cautividad nos indican que la aparición de la f. *canteneri* STGR. parece seguir un modelo de herencia para un alelo recesivo (una mutación en un simple gen, ligado al sexo), un simple locus en el cromosoma Y (gen ligado al Y) que probablemente está ausente en el cromosoma X o presente pero oculto (no activo). Lo que explicaría la ausencia de la f. *canteneri* STGR. en los ♂♂ de esta especie (nota: en contra de lo indicado por otros autores), un caso similar a las ♀♀ de *Papilio glaucus* (LINNAEUS, 1758) en Norteamérica. Finalmente mostramos algunas interesantes formas y aberraciones de *Z. rumina* (L.) obtenidas en nuestras experiencias de cría en cautividad.

**Introduction:** f. *canteneri* STGR. is a rare form of *Zerynthia rumina* (L.) which appears principally in North Africa and south of the Iberian Peninsula (its type locality). It probably appeared as a consequence of a mutation; this is to say, an inheritable change in one gene from one allelic form to another. The majority of the mutations are generally recessive. The *canteneri* form is of a yellow-ochre-orange colour (Col. pl. 7: 1C); TOLMAN & LEWINGTON (1997) indicate that this form also appears „rarely“ in the ♂♂. According to the results we have obtained, both in field observations made over a number of years, as well as those obtained from rearing in captivity, f. *canteneri* STGR. only appears in ♀♀.

The XY-XX sex-determination system is one of the most familiar and is found in *Drosophila* flies, humans, and most other mammals. In the XY sex-determination system, ♀♀ have two of the same kind of sex chromosome (XX = femaleness), while ♂♂ have two distinct sex chromosomes (XY). ♂♂ are the heterogametic (XY) sex, where Y is the sex-determining chromosome and encodes maleness. There exist genes that determine non-related sexual characters but which take precedence over sexual chromosomes. These are called sex-linked genes, and contribute to its inheritance: inheritance is therefore linked to sex.

Chromosomes X and Y are morphologically different, and so is their genetic code, so it could happen that a recessive gene situated, for example, over the X chromosome could exteriorize because of the non existence on the Y chromosome of an allele to counteract this effect; such an event wouldn't take place if the combination was XX. In human beings there are situations governed by these types of genes; well-known cases like Daltonism and haemophilia are both produced by a recessive gene linked to the X chromosome.

In genetics, the case of the fruit fly *Drosophila melanogaster* is frequently mentioned as clear example of sex-linked genes; that is, genes located on one of the sex chromosomes (X or Y) but not the other. Since, typically, the X chromosome is longer, it bears a lot of genes not found on the Y chromosome, and therefore most sex-linked genes are X-linked genes. In this fly, an X chromosome carrying a normal, dominant, red-eyed allele would be indicated by a plain X, while the recessive, mutant, white-eyed allele would be symbolized by X'. A fly with genotype XX' would normally be a ♀ with red eyes, yet be a carrier for the white-eyed allele. Because a ♂ typically only has one X chromosome, he would normally be either XY and have normal, red eyes or X'Y and have white eyes. The only way a ♀ with two X chromosomes could have white eyes is if she received an X' allele from both parents making her X'X' genotype.

A cross between a ♀ carrier and a red-eyed ♂  $XX' \times XY = XY, X'Y, XX, XX'$  Notice that while there is a „typical“ ratio of 3/4 red-eyed (XY, XX, XX') to 1/4 white-eyed, all of the white-eyed flies (X'Y) are ♂♂. These results infer that the characteristic 'colour of the eyes' is controlled by two alleles, wherein the allele which determinates the red colour is dominant over the other for the white one. If both sex and the colour of the eyes are analyzed we can verify that all the ♀♀ are red eyed, while only half of the ♂♂ are red eyed and the other half are white. The genes for the red and white colours are in the X chromosome; hence Y does not carry any gene for the colour of the eyes. ♂♂ with white eyes carry a recessive gene located in its sole X chromosome. Typically, X-linked traits show up more in males than ♀♀ because typical XY ♂♂ only have one X chromosome (Note: In accordance with the results of the current work, we will show that the canteneri form of *Z. rumina* is a totally different case than that of the fruit fly, as only one of the sexes can inherit the recessive and mutant allele), so if they get the allele on their X chromosome, they show the trait. In order for an XX ♀ to exhibit one of these X-linked traits, most of which are recessive mutations, she would need to have two copies of the allele (X'X'), which would mean that her mother would have to be a carrier and her father have the trait so she could get one allele from each of them.

The previous system of sex determinations is generally more familiar (mammals, *Drosophila*) but there are important exceptions such as in birds, butterflies, and many fish that utilize a ZZ-ZW sex determination system. Lepidopteran sex chromosomes are frequently referred to as W and Z chromosomes to emphasize their lack of homology with X and Y chromosomes (ROBINSON, 1971). The ZW sex-determination system is reversed compared to the XY system.

In the current paper, for simplicity, we use „X“ and „Y“, rather than „Z“ and „W“, to describe Lepidopteran sex chromosomes. In the Lepidoptera the  $\sigma\sigma$  have two of the same kind of chromosomes, are the XX (ZZ) sex, and  $\varphi\varphi$  are the heterogametic (XY) sex. The presence of the X chromosome induces  $\varphi$  features, while the duplication of the X chromosome induces  $\sigma$  ones. There is a closer relationship between sibling  $\sigma\sigma$  for traits located on the X-chromosome and a low heritability between mother and daughter for sex-linked traits. In Lepidoptera there is a small pseudo-autosomal region on X and Y with an obligate crossing-over at meiosis, but the most of the X chromosome (generally the Y chromosome carries less genes than the X) does not recombine (analogous to the situation in X and Y of mammals). The X-chromosome constitutes approximately 25% of the *Drosophila* genome (ASHBURNER, 1989) but only some 5% of a typical Lepidopteran genome (ROBINSON, 1971).

ALBERT & OTTO (2005) reasoned that if such a trait is encoded on the X chromosome of an XY  $\sigma$  (XY-XX System), then it incurs an immediate disadvantage because it would, by definition, only be passed onto his daughters, where it would be disadvantageous. By contrast, if the trait is encoded on the X chromosome of a XX (ZZ)  $\sigma$  (ZZ-ZW System), the trait would be passed onto sons 50% of the time. Thus, the barrier to  $\varphi$  preference evolving for antagonistic traits may be much lower in ZZ-ZW than in XY-XX Systems. In Lepidoptera, the trait gene is hemizygous in XY (ZW)  $\varphi\varphi$ , so its degree of dominance is important because a sexually antagonistic trait on the X in Lepidoptera will always be fully expressed in  $\varphi\varphi$ , while its equivalent on the XX in  $\sigma$  may be ameliorated, or even obscured, by expression of the 'normal' gene on the other X chromosome.

Our findings for the heritability of the *f. canteneri* STGR. are consistent with the theoretical predictions of a sex-linked model; the genetic component of wing's colour is primarily regulated by sex-linked genes. In the current work, we intend to determine if the genes affecting the trait are located on the X-chromosome or on the Y-chromosome. In this last case, this allele would not exist in the  $\sigma\sigma$  (XX sex). X-linkage can be relatively easy to demonstrate in Lepidoptera without sophisticated equipment, it's only necessary to rear a single generation of reciprocal hybrid crosses.

**Material and Methods:** For several generations a different number of  $\varphi\varphi$  of *Z. rumina* (L.) were randomly chosen from both the normal form and the *f. canteneri* STGR. These were taken from a biotope located in the coastal area of the province of Granada (Andalusia Region, S. Spain), where it is not difficult to find this form. The population of *Z. rumina* (L.) in this biotope seems to be genetically isolated from those of other localities situated further north of this province, where we have never observed *f. canteneri* STGR. Egg laid in captivity were obtained from these  $\varphi\varphi$ , and larvae hatched (Table 1: see column "From ova"). Pupae were conserved in a refrigerator over autumn and winter, and the wing-colouring of the adults which emerged the following spring were studied in order to determine if this form was present in  $\sigma\sigma$  and also to verify or theorize as to what kind of sex-linked inheritance affected to this species.

From the 565 live adults, some interesting and striking forms and aberrations were obtained, some of which will be subsequently commented upon.

**Results:** As the  $\varphi\varphi$  captured in nature had already partially laid their eggs, the results obtained in Table 1 are approximate, and do not match exactly to a result obtained if we had access to all of the eggs. As the percentage between  $\sigma\sigma$  and  $\varphi\varphi$  of each brood is approximately 50% (this is normal), the remaining results obtained are considered significantly relevant

Period	From ova: $\sigma\sigma + \text{♀♀}$	Total $\sigma\sigma$ (all N)	Total (♀♀ N+C)	♀♀ C from total ♀♀	♀♀ N from total ♀♀
Offspring from ova <i>canteneri</i> ♀♀					
2005-2006	80	38 47,50%	42 52,50%	40 95,24%	2 4,76%
2003-2004	50	28 56,00%	22 44,00%	17 77,27%	5 22,73%
2001-2002	140	66 47,14%	74 52,86%	49 66,22%	25 33,78%
2000-2001	120	62 51,67%	58 48,33%	40 68,97%	18 31,01%
Offspring from ova normal ♀♀					
2005-2006	73	38 52,05%	35 47,95%	15 42,86%	20 57,14%
2004-2005	102	49 48,04%	53 51,96%	21 39,62%	32 68,38%

Table 1.-Offspring from ova of *f. canteneri* STGR. and normal ♀♀ (N=normal;C=*canteneri*)

In Table 1, periods: 2003-2004; 2001-2002 and 2000-2001, from ova of *f. canteneri* STGR. ♀♀, we can see similar results in the descendants of the three ♀♀: From a total of adults obtained (of 50, 140 and 120), approximately 50% of ♀♀ hatched (ranging from 44% to 52%), of which between 66,22 and 77,27 percent were found to correspond to *f. canteneri* STGR. If, in principle, we assume the hypothesis that their heredity is determined by autosomic genes (Note: At the end of this paper this possibility is discarded) in the X chromosome, theoretically the most favourable combination would be [abbreviations: W = without allele in Y chromosome; Z = dominant allele (normal wing colour) in the X chromosome; z = recessive allele (*canteneri* wings colour) in the X chromosome; ZW = normal ♀; zW = *canteneri* ♀ chromosome]: Parents: Zz x zW = Zz, zz, ZW, zW. This is to say that 50% of the ♀♀ would be normal while the remaining 50% would be *f. canteneri* STGR., which matches approximately to our findings; but in accordance with this combination, this does not coincide exactly because zz males should have appeared (homozygous for the recessive allele, in other words *canteneri*). Conclusively this is not the case (autosomic genes), because all the 281  $\sigma\sigma$  obtained were normal and none of them were *f. canteneri* STGR. For the period 2005-2006, eggs were also taken from a *f. canteneri* STGR. ♀, when we obtained 80 adults, of which 42 (52,50%) were ♀♀. Almost all of these (40) were *f. canteneri* STGR. (95,24%), a surprising result when compared with the previous periods mentioned. As in the previous case, we do not know if the results would have been similar to these if we had been able to obtain all of the egg stack.

We can also see in Table 1 that the percentage of ♀♀ and  $\sigma\sigma$  hatched from the eggs laid by ♀♀ with a normal phenotype were also similar (approximately 50%). During the period 2005-2006, of 35 ♀♀ in total, 42,86% were *f. canteneri* STGR., the remainder (57,14%) were of the normal form. During the period 2004-2005, with a major number of specimens hatched (102), the result was similar to the previous period mentioned: 39,62% of the ♀♀ were *f. canteneri* STGR. and the remainder were of the normal form. In both of these similar cases, no  $\sigma\sigma$  with the *canteneri* phenotype were obtained.

By means of the 565 samples studied, we have observed that the heritability of the *f. canteneri* STGR. is consistent with the theoretical predictions of a sex-linked model, in that the genetic component of wing's colour is regulated by sex-linked genes. In the case of *Z. rumina* (L.), the genes affecting the trait are not located on the X-chromosome because of the absence in the  $\sigma\sigma$  of the *canteneri* phenotype; these would exist if the determinant genes of this trait

were located on the X chromosome. As indicated in the introduction, in TOLMAN & LEWINGTON (1997) it is stated, in our opinion incorrectly, that *f. canteneri* STGR. can also appear in the  $\sigma\sigma$ . In the biotope where the reared material was taken from, during the past decade we have never seen a single  $\sigma$  with a similar colouration to *f. canteneri* STGR. In other southern localities in the south Iberian Peninsula where *Z. rumina* (L.) is found, where  $\text{♀♀}$  of *f. canteneri* STGR. have never been found (!). We have only observed one or two  $\sigma\sigma$  specimens with a wings slightly orange coloured, or in any way ascribable to such a form: this is confirmed by the fact of that there is a total absence of  $\text{♀♀}$  of the *canteneri* phenotype.

We believe that the ♀ colour polymorphism heredity in *Z. rumina* (L.) is similar to the case of the North American species *Papilio glaucus* (L.). In this species,  $\text{♀♀}$  are yellow or melanic (where most of the yellow colour is replaced by black). As in Lepidoptera,  $\text{♀♀}$  are heterogametic XY and  $\sigma\sigma$  homogametic XX. The genetic analysis of *P. glaucus* (L.) therefore suggests that melanism is controlled by a single locus on the Y chromosome that is either absent from the X or present but suppressed (NIJHOUT, 1991). This would explain the absence of the melanic form in the  $\sigma\sigma$  [in our case the absence of *f. canteneri* STGR. in the  $\sigma\sigma$  of *Z. rumina* (L.)]. Interestingly, SCRIBER et al. (1996) described a single major sex-linked gene (the Y-linked 'black') and a modifying gene (the X-linked 'suppressor') which suppresses the melanic phenotype in hybrid crosses to the closely related *Papilio canadensis* (ROTHSCHILD & JORDAN, 1906).

Generally, the dominant alleles direct the synthesis of the functional enzymes and the recessive alleles, the non-functional enzymes. The synthesis of certain enzymes influence the occurrence of certain pigments which define the phenotype's colour. The pigments that underlie these colours belong to a range of different chemical classes: pteridines forming white, yellow or red; ommatins forming red or red-brown, and melanins forming grey or black (NIJHOUT, 1980; KOCH, 1992). Interestingly, some pigments are only found in certain groups of Lepidoptera, for example the yellow papiliochromes discussed here are exclusive to the papilionids (UMEBACHI, 1985).

In the *Z. rumina* (L.) populations, where the recessive mutant allele exists which defines *f. canteneri* STGR., this allele is situated on chromosome Y where it conducts the synthesis of certain enzymes (together with other unknown factors, perhaps some hormonal influence), catalysing the required reactions for producing a canteneri phenotype. The lack of the chromosome Y in the  $\sigma\sigma$  therefore this allele is absent and does not act. This modification of multiple enzyme activities in concert is consistent with a single Y-linked (female) factor.

### Some interesting forms and aberrations in *Z. rumina* (L.)

From the total of 565 specimens obtained, 281  $\sigma\sigma$ , 284  $\text{♀♀}$  [182 *f. canteneri* STGR.; 102 *rumina* (L.) phenotype], we also obtained some interesting aberrations or forms (independently of their normal or *canteneri* colour in the wings) of which we shall show the most striking.

Within the aberrations, in colour pl. 7: 2 the specimen 'a' is a  $\sigma$  with normal colouring; on the forewing, some patterns - and almost all the red colour - are missing, in others the patterns are totally merged, resulting in an almost melanic wing. Specimens 'b' and 'c' are  $\text{♀♀}$ , and their colour is very similar to *f. canteneri* STGR., looking very much like specimen 'a'; in the hindwings there are several patterns merged to a certain degree. Fig. 3 (col. pl. 7) is a strange aberration: it has a *canteneri* ground colour, but the postdiscal and submarginal areas of the forewings and hindwings are hyaline, semi-transparent, and the underside is much the same.

**Some forms:** In colour pl. 7: 4, looking at the  $\sigma\sigma$  from left to right, a specimen lacking some patterning or black markings with no red spots; two specimens with extremes of black markings; the last specimen has the red spotting greatly developed, especially in the central area of the wing. In colour pl. 7: 5 we can see two  $\text{♀♀}$  of the *canteneri* phenotype: the specimen 'A' is typical *canteneri*, while on specimen 'B' the black markings are much more developed, replacing the red spots which are almost absent. In colour pl. 7: 6 we can observe in two *canteneri*  $\text{♀♀}$  an asymmetric patterning between the right and the left forewings. In Fig. 7 we see an exceptional *canteneri*  $\text{♀}$ : the black markings (two in each forewing) are encircled with yellow, looking much like a "mask", and the black markings are highly developed, especially in the discal area of the forewings. In colour pl. 7: 8 is a  $\text{♀}$  with a normal ground colour, with some unusual red spots in the marginal areas of the hindwings.

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# Colour plate 7/ Farbtafel 7

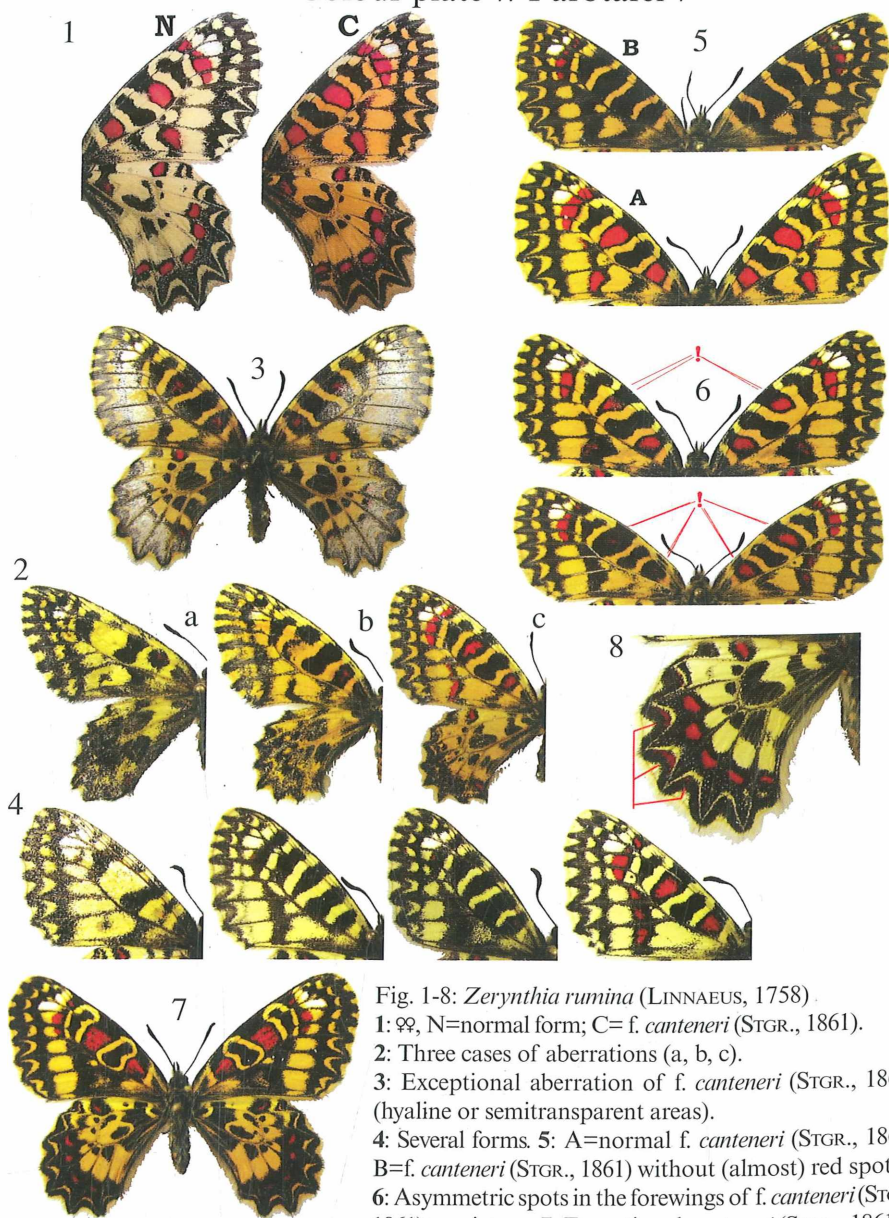


Fig. 1-8: *Zerynthia rumina* (LINNAEUS, 1758).

1: ♀, N=normal form; C= f. *canteneri* (STGR., 1861).

2: Three cases of aberrations (a, b, c).

3: Exceptional aberration of f. *canteneri* (STGR., 1861). (hyaline or semitransparent areas).

4: Several forms. 5: A=normal f. *canteneri* (STGR., 1861); B=f. *canteneri* (STGR., 1861) without (almost) red spots.

6: Asymmetric spots in the forewings of f. *canteneri* (STGR., 1861) specimens. 7: Exceptional *canteneri* (STGR., 1861).

8: Form (red in marginal area).